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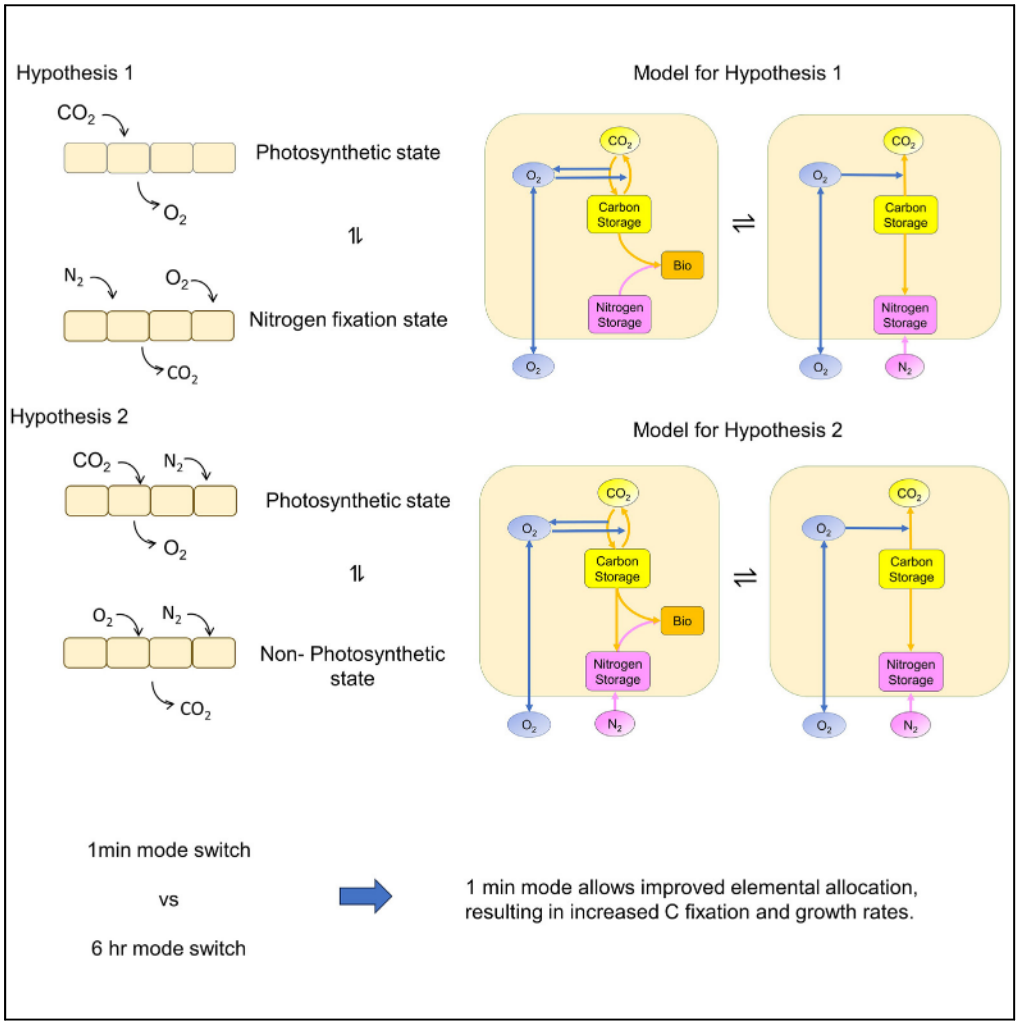

Meng Gao, Jamal Andrews, Gabrielle Armin, Subhendu Chakraborty, Jonathan P. Zehr, Keisuke Inomura

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Highlights

O_2 levels in *Trichodesmium* can decrease rapidly at the start of N_2 fixation states

The rapid mode switching allows faster cellular growth than the slower mode

The faster growth is due to improved C and N allocation in cells

Our study provides a mechanistic understanding of aerobic daytime N_2 fixation

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Rapid mode switching facilitates the growth of *Trichodesmium*: A model analysis

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SUMMARY

***Trichodesmium* is one of the dominant dinitrogen (N_2) fixers in the ocean, influencing global carbon and nitrogen cycles through biochemical reactions. Although its photosynthetic activity fluctuates rapidly, the physiological or ecological advantage of this fluctuation is unclear. We develop a metabolic model of *Trichodesmium* that can perform daytime N_2 fixation. We examined (1) the effect of the duration of switches between photosynthetic and non-photosynthetic cellular states and (2) the effect of the presence and absence of N_2 fixation in photosynthetic states. Results show that a rapid switch between photosynthetic and non-photosynthetic states increases *Trichodesmium* growth rates by improving metabolic efficiencies due to an improved balance of C and N metabolism. This provides a strategy for previous paradoxical observations that all *Trichodesmium* cells can contain nitrogenase. This study reveals the importance of fluctuating photosynthetic activity and provides a mechanism for daytime N_2 fixation that allows *Trichodesmium* to fix N_2 aerobically in the global ocean.**

INTRODUCTION

Trichodesmium is a cyanobacterial genus whose species have a multicellular filamentous morphology[1] and are widely distributed in tropical and subtropical areas.[2–7] It is also an important dinitrogen(N_2)-fixing cyanobacterial genus in the global ocean.[8,9] In addition to fixing N_2, *Trichodesmium* is photosynthetic and evolves oxygen (O_2).[10,11] *Trichodesmium* plays an important role in ocean biogeochemical cycles[12] since it contributes to carbon (C) and nitrogen (N) cycling. Due to its ecological importance, many laboratory experiments[13–15] and modeling studies[3] have been performed to understand its physiology, including N_2 fixation strategies.[4,5,16]

The N_2-fixing enzyme (nitrogenase) with metal cofactors can be inactivated by presence of O_2.[17,18] Since cyanobacteria evolve O_2 through oxygenic photosynthesis, they have to use physiological or morphological strategies to avoid the inactivation of nitrogenase.[16,17,19] Several strategies in cyanobacteria have been reported,[17,19] for example, the formation of specialized N_2 fixation cells that lack oxygenic photosynthetic activity (heterocysts).[16,20–22] However, *Trichodesmium* does not form heterocysts.[23] Although a recent study found that *Trichodesmium* can fix N_2 in the dark,[24] many previous studies suggest it appears to fix N_2 and photosynthesize in the same cells during the daytime with light.[25–28] It is still unresolved how *Trichodesmium* fixes N_2 aerobically in the light while evolving photosynthetic O_2.

Although *Trichodesmium*'s N_2 fixation mechanism is still unclear, results of previous studies suggested that photosynthetic activities can be regulated during cellular-level photosystem state transitions, which are on the order of 1 minute.[3,16,29,30] Based on this photosynthesis 1-min on/off switch, we developed two hypotheses: (H1) nitrogen fixation only occurs during a non-photosynthetic state, or (H2) *Trichodesmium* continues fixing N_2 during photosynthesis.

As for H1, a model by Inomura et al. 2019[3] suggested that the intracellular O_2 may be decreased rapidly on the timescale of seconds without photosynthesis, which provides an opportunity for N_2 fixation. The rapid recovery of nitrogenase can be supported by evidence from studies in other species, which have a protein (Shethna Protein II, FeSII) for conformational protection of nitrogenase.[31] This protein can quickly respond to O_2 and make the nitrogenase in an inactive but oxygen-tolerant state until recovery. However, this protein (or its genes) has not been found in *Trichodesmium*, and reactivation of nitrogenase likely requires a much longer time than seconds to recover from inhibition.[32,33] This argument leads to Hypothesis 2 (H2), which states that *Trichodesmium* may continue fixing N_2 during photosynthesis. Studies show that under ambient oxygen concentrations, it takes more than a few minutes to deactivate nitrogenase.[31,33] Thus, *Trichodesmium* might tolerate short time intervals at high O_2 concentrations, for example, 1 minute. It takes tens of minutes to resynthesize nitrogenase,[34,35] which excludes the possibility that cells are constantly resynthesizing nitrogenase if it is damaged; and repair cannot keep pace with inactivation at those short time intervals.

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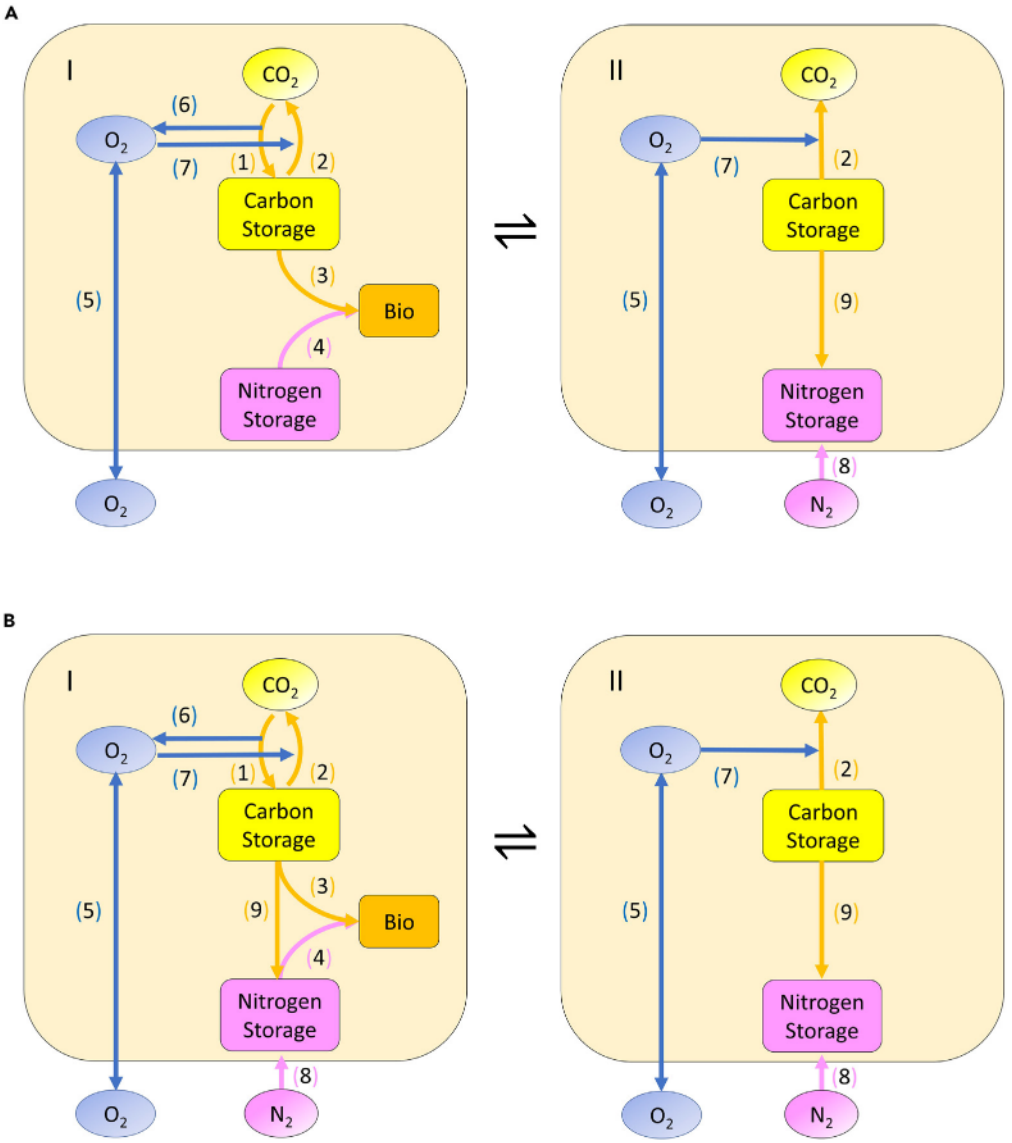

Figure 1. Schematic depiction of molecular pools and fluxes in the model

(A) H1: Photosynthesis and N_2 fixation occur at different times.

(B) H2: Photosynthesis and N_2 fixation occur simultaneously. For (A) and (B): (I) Photosynthetic state. (II) Non-photosynthetic (N_2 fixation) state. The symbol ⇌ represents state transition. Pathways: (1) C fixation. (2) Respiration. (3) C consumption in growth. (4) N consumption in growth. (5) O_2 diffusivity. (6) O_2 production in carbon fixation. (7) O_2 consumption in respiration. (8) N_2 fixation. (9) C consumption in N_2 fixation (for energy and electron). Different colors represent different element pools and fluxes: orange arrows and yellow items are for C, pink items are for N, and blue items are for O_2. The orange rounded corner rectangles mean biomass. The cream rounded corner rectangles mean *Trichodesmium* cells. According to the previous observation of *Trichodesmium*,[30] we switched these two states every minute and every 6 hours. We ran the models for 12 hours. And we assumed that there was no growth in the non-photosynthetic state.

Based on these hypotheses, we built a metabolic model of *Trichodesmium* (Figure 1) and ran it under two situations (H1 and H2) and two switch modes (1-min: switch every minute and 6-h: switch every 6 hours) to answer three main questions: (1) How do the cells fix N_2 even though they are producing O_2? (2) How can mode switching influence growth rate and why? (3) Based on element (C and N) allocation, which switch mode is more efficient? The following model illustrates the mechanism of *Trichodesmium* N_2 fixation and the associated advantages of the mechanism.

RESULTS AND DISCUSSION

Overview of the model

We developed a physiological model of a *Trichodesmium* cell that performs photosynthesis to obtain carbon (C) and fix N_2 to obtain nitrogen (N). The cell produces O_2 during photosynthesis and uses both C and O_2 to maintain respiration. Based on previous experimental evidence,[30]

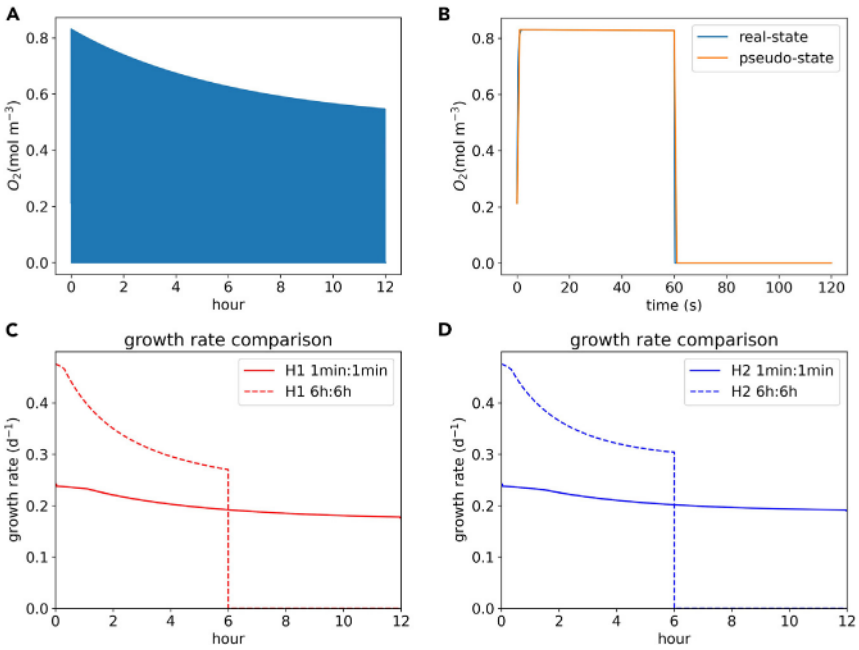

Figure 2. O_2 level and growth rate changes in 12 h

(A) Changes in O_2 concentration in 12 h (H2, and H1 pattern is similar in Figure S1).

(B) Changes in O_2 concentration in 2 min (H2, and H1 pattern is similar in Figure S1); 60 s for photosynthetic state and 60 s for non-photosynthetic state. Pseudo-state means we used steady-state conditions (O_2 values do not change) for the simulation.

(C) Growth rate comparison of different modes in H1.

(D) Growth rate comparison of different modes in H2. For (C) and (D), in the rapid switch mode, we plot the growth rate by taking an average of 2 min.

here we assume that the cell can switch between photosynthetic and non-photosynthetic states. Accordingly, we built and examined two hypotheses H1 and H2; H1: N_2 fixation happens only during the non-photosynthetic state (Figure 1A, Video S1), whereas H2: N_2 fixation happens in both photosynthetic and non-photosynthetic states (Figure 1B, Video S2). Moreover, we considered two different switch modes based on the time duration of each state: a rapid mode, where the switching between two states happens every minute (1-min mode), and a slow mode, where the switching happens every 6 hours (6-h mode). To calculate cellular element dynamics (C, N, O_2), we resolved molecular transport, photosynthesis, respiration, biosynthesis, and N_2 fixation as the critical pathways. The following are the most important results.

O_2 fluctuation analysis

Our results show that O_2 concentrations change dramatically as the state switches in both hypotheses. Figure 2A and 2B show that it took less than 1 s during the daytime to reach a steady O_2 concentration after switching to the non-photosynthetic (N_2 fixation) state. In the photosynthetic state, the O_2 reached a high level, while in the N_2 fixation state, O_2 concentration decreased and remained low. The rapid decrease of O_2 in the N_2 fixation state might provide conditions to allow nitrogenase activity. Nitrogenase might (H1) reactivate during low O_2 conditions or (H2) tolerate the high O_2 level for a short time during the photosynthetic state. Based on these rapid O_2 changes, *Trichodesmium* can photosynthesize and fix nitrogen (Figure S2) in the same cell, even with the temporal separation of only 1 minute.

Our simulated quick changes in O_2 concentrations are similar to those of a previous modeling study,[3] which estimated an extremely short residence time of O_2 (the time to consume all O_2 by respiration) in *Trichodesmium* cells on the order of 1 s. The rapid decrease results from the high respiration rate, as suggested by previous studies.[3,23,36,37] Since N_2 fixation requires substantial energy in the form of ATP (16 ATP per N_2 fixed), it needs to be coupled with high aerobic respiration rates to provide ATP. The high aerobic respiration rates could explain our results that the intracellular O_2 level changes rapidly, and that the O_2 concentration is low throughout the N_2 fixation state. In *Trichodesmium*, increased respiration in a cell might also reduce the plastoquinone pool and transmit negative signals to photosystem II (PSII), which would decrease photosynthesis and consequently the production of O_2.[10,36] In addition to respiration, previous studies of N_2-fixers suggested that lower O_2 levels can also be maintained in the cell[37,38] by lowering O_2 diffusivity[3] with the use of multiple membrane layers (gram-negative bacterium)[39] and extracellular polymeric substances (EPS)[40–44] (*Azotobacter vinelandii* and *Trichodesmium*), as well as using an alternative electronic transfer (AET) pathway (*Trichodesmium*).[36]

Growth rate comparison

We compared growth rates in 12 h of daytime (with light) between rapid mode switching and slow mode switching (Figure 2C and 2D). For both H1 and H2, the average growth rates over 12 h for rapid mode (H1: 0.20 day^{-1}, H2: 0.21 day^{-1}, hereafter we use d^{-1} to represent

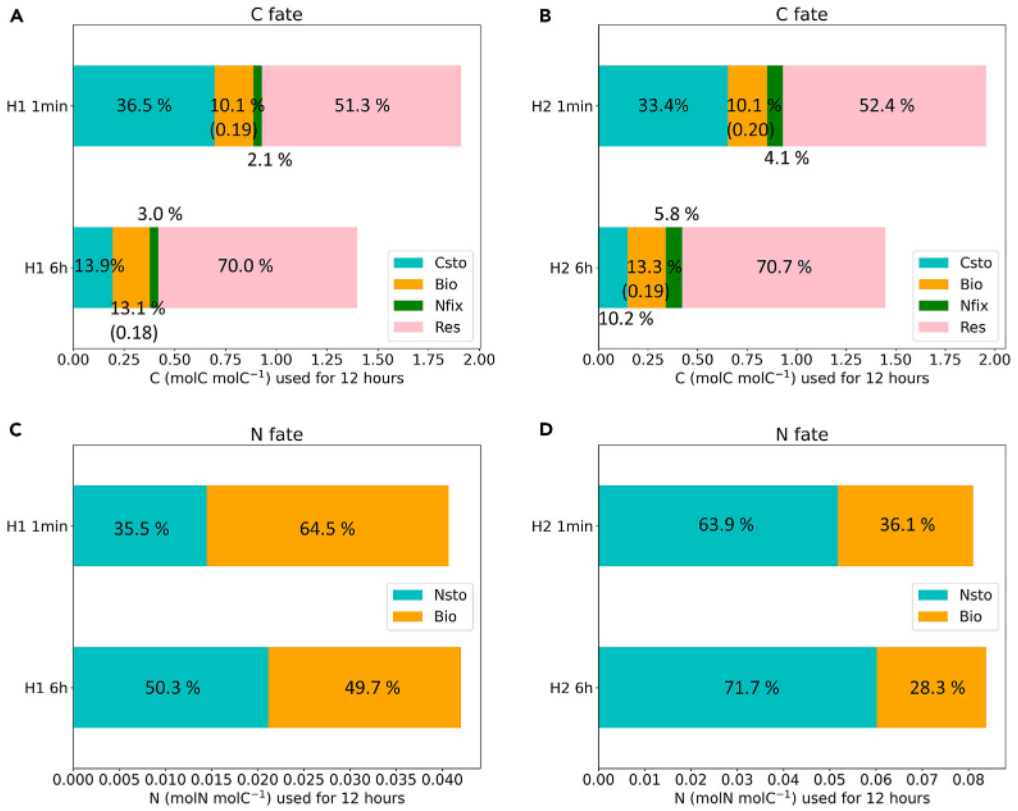

Figure 3. Element fate

C fate (C usage distribution in 12 h) comparison for H1 (A) and H2 (B). N fate (N usage distribution in 12 h) comparison for H1 (C) and H2 (D). Csto means C storage, Bio means element (C or N) used in growth, Nfix means C used in N_2 fixation, Res means C used in respiration, and Nsto means N storage, values in figures mean percentage values, and values in parentheses and the stack length mean the absolute values.

day^{-1}) are higher than those of slow mode (H1: 0.17 d^{-1}, H2: 0.18 d^{-1}). Figure 2C and 2D show that under H1 and H2, the first 6-h growth for the rapid mode is lower, but the remaining 6-h growth rate is higher. The difference between these two modes suggests that the rapid switch with higher average growth rates is a better strategy for *Trichodesmium*, which results from a better element supply and distribution inside the cell. To determine the reason for the higher growth rate, we simulated element fate.

Element fate and comparison

Here we compared C and N fates for two switching modes (Figure 3). During the rapid mode, under H1 and H2, the cell used a larger percentage of C in storage and less in respiration (Figure 3A and 3B). This indicates that in the rapid mode, less percentage of C can be released with respiration, but more C can stay in the cells for metabolisms. Regarding absolute values (indicated in x-labels), the total amount of C invested in cellular processes (including growth) is higher in the rapid mode irrespective of H1 and H2. As for the N fate, results for both hypotheses showed that the rapid mode utilized more N in growth, in terms of both percentage and absolute values, than N storage (Figure 3C and 3D). The total utilization of N for the two modes is slightly different (H1: 0.041 and 0.042, H2: 0.081 and 0.084, unit: mol N mol C^{-1}).

We can explain our growth rate results based on the element comparison. To maintain the higher growth rate during the rapid mode switching, more N is used for growth (biomass) and less C percentage is used for respiration. This shows that the rapid mode switching is a more efficient strategy with faster growth and less C lost. Thus, *Trichodesmium* evolved the rapid mode switching to improve benefits from photosynthesis and N_2 fixation.

In summary, *Trichodesmium* can switch every minute between photosynthesis and N_2 fixation to achieve temporal separation. This can happen because the O_2 levels change so fast that nitrogenase can be protected during the N_2 fixation state. The rapid mode change increases the growth rate by improving the distribution of C and N usage in cells and makes *Trichodesmium* a very successful N_2-fixer and primary producer in the ocean and dominates the phytoplankton community in some areas.[5,45–49]

Comparison to previous studies and implications for future work

Previous research suggested that both spatial and temporal separation mechanisms were used in *Trichodesmium*. Studies reported that *Trichodesmium* could form specialized cells (termed diazocytes) where N_2 fixation was localized.[15] However, it is still controversial since

several studies reported that nitrogenase is randomly distributed in *Trichodesmium* cells or even in all cells, and a modeling study found that spatial separation is unnecessary.[36] As a result, temporal separation is thought to be more necessary. Rapid state transition switches were shown in cellular-level fluorescence kinetics experiments.[29,30] Our study reconciles these observations and provides possible mechanisms that facilitate daytime N_2 fixation. Our study not only elucidates *Trichodesmium*'s O_2 protection mechanisms but also reveals a strategy for non-heterocyst forming and daytime N_2-fixing cyanobacteria, which can be a model for mechanisms in other species in addition to *Trichodesmium*. This model may provide a metabolic module of *Trichodesmium* for existing ecological models with *Trichodesmium*[50–52] for predicting their physiological response to the environment and the consequent ecological and biogeochemical impacts.

In our results, rapid mode switching results in higher efficiency in terms of resource use with more N in growth and less C percentage in respiration compared to slow mode switching. Our simulated growth rates are consistent with experimentally observed *Trichodesmium* growth rates (0.1–0.5 d^{-1}) in previous studies.[3,53,54] In addition, our simulated nitrogen fixation rates (Figure S2) are in a reasonable scale compared to previous observations (0.006–0.569 mol N mol C^{-1} d^{-1}).[11,55,56] Comparing our simulated average N_2 fixation rate and growth rate data with previous studies results (Figure S3), we found that our model is reasonable, and our results are similar with the real condition. Interestingly, nitrogenase can also be regulated by several environmental factors, and thus environmental factors may be important in affecting *Trichodesmium* N_2 fixation and growth in the ocean. For example, ocean acidification[57,58] and nutrients like iron[57,59] have been found to influence nitrogenase efficiency. Future research on how environmental factors (e.g., ocean pH, and nutrients like iron and phosphorus) affect nitrogenase and state transitions will be explored, which is important for predicting the response of *Trichodesmium* to climate change.

Limitations of the study

The simulated results of the model show the possibility and advantages of the rapid switching mechanisms. However, more experimental studies and observations are needed to show more evidence. In this study, we set a simplified average initial condition and have not considered how different environmental conditions influence the state transition, growth rate, and element allocation. Potential further studies can focus on highly different nutrient conditions (e.g., N or P limitation) that can influence the metabolisms. Although our model is general, some necessary changes will be required for applying it to other N_2 fixers, e.g., for heterocyst N_2 fixers like *Rachelia*, which involves different controlling mechanisms for O_2. Additionally, adjustments to parameters will also be necessary when applying this model to other organisms.

Conclusion

With a mechanistic model of *Trichodesmium*, we found that rapid mode switching could facilitate N_2 fixation because cellular O_2 concentrations can decrease to a very low level in 1 second. This switch mode can also explain why most *Trichodesmium* cells contain nitrogenase and how they can fix N_2 during the day. The rapid mode switching also keeps C and N concentrations flexible in cells to improve C and N allocation, thus facilitating the growth of *Trichodesmium*. Our results show that switching mode rapidly is an efficient strategy for the growth of non-heterocyst-forming cyanobacteria and daytime N_2-fixers, which can guide further study in ocean N_2-fixers.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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  - Model simulation
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- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2024.109906.

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AUTHOR CONTRIBUTIONS

Conceptualization: M.G., K.I., and J.P.Z.; Data curation: M.G.; Formal Analysis: M.G. and K.I.; Fundings acquisition:K.I. and J.P.Z.; Investigation: M.G. and K.I.; Methodology: M.G., J.A., and K.I.; Project administration: K.I.; Resources: M.G., J.A., G.A., K.I., S.C., and J.P.Z.; Software: M.G.; Supervision: K.I.; Visualization: M.G., J.A., K.I., and S.C.; Writing – original draft: M.G., G.A., and K.I.; Writing – review and editing: M.G., G.A., K.I., S.C., and J.P.Z. All authors have read and agreed to the published version of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Simulated data	This paper	[Tricho_Gao_2024]: https://doi.org/10.5281/zenodo.8062145
Experimental test data	Inomura et al. 2020[60]	https://doi.org/10.3390/plants9020192
Software and algorithms		
Python code for the model	This paper	[Tricho_Gao_2024]: https://doi.org/10.5281/zenodo.8062145

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Meng Gao (meng_gao@uri.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- All the simulated and experimental data have been deposited at [Tricho_Gao_2024] and are publicly available as of the date of publication. DOIs are listed in the key resources table.
- All original code has been deposited at [Tricho_Gao_2024] and is publicly available as of the date of publication. DOIs are listed in the key resources table.
- "Any additional information required to reanalyze the data reported in this work paper is available from the lead contact upon request."

METHOD DETAILS

Here, we describe the ecological model and calculation in detail. Our model includes the photosynthetic state and non-photosynthetic (N_2 fixation) state of marine phytoplankton *Trichodesmium* (Figure 1). We calculated cellular C, N, and O_2 concentrations during the half of the diurnal cycle using diffusivity, photosynthesis, respiration, N_2 fixation, and biosynthesis. Based on our two hypotheses, photosynthesis and N_2 fixation can happen at the same time in H2, whereas they are separated by time in H1. In the following, first, we describe photosynthetic states for the two hypotheses and then the N_2 fixation states. The following are the equations we used to build the model.

Photosynthetic state

H1. To model the phytoplankton C changing rate ($\frac{dC_{sto}}{dt}$) (Equation 1) in the photosynthetic state, we assumed that it could be described as the difference between the C fixing rate (F_{cfix}) and C consuming rate. This consumption includes respiration (F_{Bio} E) and growth (F_{Bio}), where E is the ratio of respiration to biomass production.

$$\frac{dC_{sto}}{dt} = F_{cfix} - F_{Bio}(1 + E). \quad \text{(Equation 1)}$$

To estimate cellular O_2 flux ($\frac{d[O_2]}{dt}$), we included diffusivity, photosynthesis, and respiration pathways (Equation 2). We used a product of diffusivity coefficient (A) and O_2 difference ($[O_2]_E - [O_2]$) to represent the O_2 change in diffusivity between the extracellular (E) and intracellular environment. ρ_C^{Bio} and $Y_{Cfix}^{O_2:C}$ are cell carbon density and O_2 to C ratio in photosynthesis. $Y_{res}^{O_2:C}$ mean O_2 to C ratio in respiration. Here we used them to quantify the O_2 change in carbon fixation (F_{cfix}) and respiration ($F_{Bio}E$).

$$\frac{d[O_2]}{dt} = A([O_2]_E - [O_2]) + F_{cfix}\rho_C^{Bio}Y_{Cfix}^{O_2:C} - F_{Bio}E\rho_C^{Bio}Y_{Res}^{O_2:C}. \quad \text{(Equation 2)}$$

Next, we calculated N flux ($\frac{dN_{sto}}{dt}$) (Equation 3) which only changes due to the consumption of nitrogen in growth in the photosynthetic state of *Trichodesmium*. Here, we used growth (F_{Bio}) times the N to C ratio in cells ($\frac{(Y_{Bio}^{N:C}+N_{sto})}{(1+C_{sto})}$). In this term, $Y_{Bio}^{N:C}$ represents N to C ratio in biomass.

We can also describe it as the N concentration when we use mol N mol C^{-1} as the unit of N content. We added the amount of N in the storage, N_{sto}, to it to calculate the whole N concentration ($Y_{Bio}^{N:C} + N_{sto}$). Here we used ($1 + C_{sto}$) to represent the total C concentration in the cell. 1 here means the original functional C in cells and C_{sto} means C storage in cells.

$$\frac{dN_{sto}}{dt} = -F_{Bio}\frac{(Y_{Bio}^{N:C}+N_{sto})}{(1+C_{sto})}. \quad \text{(Equation 3)}$$

In Equation 4, we assumed that the C fixation rate (F_{cfix}) could increase with light intensity (I) but becomes saturated when it reaches a maximum (F_{Cfix}^{max}). Here, A_i represents the light saturation coefficient.

$$F_{Cfix} = F_{Cfix}^{max}\left(1 - e^{-A_i I}\right). \quad \text{(Equation 4)}$$

In Equation 5, we considered C and N cellular concentrations as two factors limiting biomass production. Due to Liebig's law of minimum, growth is determined by the scarcest factors. Besides, biomass production can increase with C and N concentrations but will reach saturation at a high value. As a result, the form of the equation resembles Monod kinetics. Therefore, we calculated the actual biomass production, F_{Bio}, using the minimum of available C ($\frac{C_{sto}}{C_{sto}+K_C}$), and N ($\frac{N_{sto}}{N_{sto}+K_N}$) for biosynthesis. Here, K_C means the half-saturation concentration for C and K_N means the half-saturation concentration for N.

$$F_{Bio} = F_{Bio}^{max}\, min\left(\frac{C_{sto}}{C_{sto}+K_C}, \frac{N_{sto}}{N_{sto}+K_N}\right) \quad \text{(Equation 5)}$$

In Equation 6, we calculated growth rates (μ) based on biomass production (F_{Bio}). And we divide it by ($1 + C_{sto}$), which means total C in cells to transfer the unit. This growth rate equation can be used for all the states we discuss here.

$$\mu = \frac{F_{Bio}}{1+C_{sto}} \quad \text{(Equation 6)}$$

H2. Here N_2 fixation happens during photosynthesis, and thus we modified Equations 1, 2, and 3 accordingly:

To calculate the C changing rate, we added N_2 fixation as another source of expenditure of C (Equation 7). We included two more terms: $F_{N_2fix}Y_{N_2fix}^{C:N}$ and $F_{N_2fix}Y_{N_2fix}^{N:O_2}Y_{Res}^{C:O_2}$, which respectively mean the C usage in N_2 fixation and the C usage in respiration of N_2 fixation. Here, F_{N_2fix} means N fixation rate, $Y_{N_2fix}^{C:N}$ represents C to N ratio in N_2 fixation, $Y_{N_2fix}^{N:O_2}$ represents a conversion factor from N to O_2 in N_2 fixation, and $Y_{Res}^{C:O_2}$ means C to O_2 ratio in respiration.

$$\frac{dC_{sto}}{dt} = F_{cfix} - F_{Bio}(1+E) - F_{N_2fix}Y_{N_2fix}^{C:N} - F_{N_2fix}Y_{N_2fix}^{N:O_2}Y_{Res}^{C:O_2} \quad \text{(Equation 7)}$$

To revise the O_2 flux equation, we subtracted a term ($F_{N_2fix}\rho_C^{Bio}Y_{N_2fix}^{N:O_2}$) representing the respiratory cost of O_2 during N_2 fixation (Equation 8).

$$\frac{d[O_2]}{dt} = A([O_2]_E - [O_2]) + F_{cfix}\rho_C^{Bio}Y_{Cfix}^{O_2:C} - F_{Bio}E\rho_C^{Bio}Y_{Res}^{O_2:C} - F_{N_2fix}\rho_C^{Bio}Y_{N_2fix}^{N:O_2} \quad \text{(Equation 8)}$$

To revise the N flux equation, we added a N_2 fixation term ($F_{N_2fix}^{max}\left(\frac{C_{sto}}{C_{sto}+K_C}\right)$) (Equation 9), which depends on cellular C storage (C_{sto}). We assume that N_2 fixation increases with increasing C storage and saturates due to physiological constraints to the maximum limit of N_2 fixation rate ($F_{N_2fix}^{max}$).

$$\frac{dN_{sto}}{dt} = -F_{Bio}\frac{(Y_{Bio}^{N:C}+N_{sto})}{(1+C_{sto})} + F_{N_2fix}^{max}\left(\frac{C_{sto}}{C_{sto}+K_C}\right) \quad \text{(Equation 9)}$$

Non-photosynthetic (N_2 fixation) state

This state is the same under both the hypotheses H1 and H2. To calculate C changing rate in the N_2 fixation state, we assumed that it could be affected by two pathways: N_2 fixation ($F_{N_2fix}Y_{N_2fix}^{C:N}$) and respiration ($F_{Res}/Y_{Res}^{O_2:C}$). Here, F_{N_2fix} and F_{Res} represent N_2 fixation rate and respiration rate, respectively. We use C to N ratio ($Y_{N_2fix}^{C:N}$) in N_2 fixation and O_2 to C ratio ($Y_{Res}^{O_2:C}$) in respiration to transform the units.

$$\frac{dC_{sto}}{dt} = -F_{N_2fix}Y_{N_2fix}^{C:N} - F_{Res}\Big/\left(Y_{Res}^{O_2:C}\rho_C^{Bio}\right) \quad \text{(Equation 10)}$$

We calculate respiration rate (F_{Res}) in Equation 11 by assuming that it increases with cellular O_2 concentration ($[O_2]$) and can reach a maximum rate (F_{Res}^{max}).

$$F_{Res} = F_{Res}^{max}\left(\frac{[O_2]}{[O_2]+K_{O_2}}\right) \quad \text{(Equation 11)}$$

Here, K_{O_2} means half-saturation concentration of O_2.

The only pathway affecting the N changing rate ($\frac{dN_{sto}}{dt}$) is N_2 fixation ($F_{N_2 fix}$), which is calculated in Equation 12. N_2 fixation depends on cellular C storage (C_{sto}). We assume that N_2 fixation increases with increasing C storage and saturates due to physiological constraints to the maximum limit of N_2 fixation rate ($F^{max}_{N_2 fix}$) as:

$$\frac{dN_{sto}}{dt} = F_{N_2 fix} = F^{max}_{N_2 fix}\left(\frac{C_{sto}}{C_{sto}+K_C}\right), \quad \text{(Equation 12)}$$

where K_C is the half-saturation constant of C storage.

The cellular O_2 flux (Equation 13) is obtained by subtracting the respiratory consumption from the diffusive O_2 input:

$$\frac{d[O_2]}{dt} = A\left([O_2]_E - [O_2]\right) - F^{max}_{Res}\left(\frac{[O_2]}{[O_2]+K_{O_2}}\right) \quad \text{(Equation 13)}$$

We calculated all of the element dynamics in the two states by simplification of the Taylor Expansion, expressed by:

$$Con(t+\Delta t) = Con(t) + \frac{dCon}{dt}\Delta t \quad \text{(Equation 14)}$$

where Con is the concentration of C, N, or O_2 and $\frac{dCon}{dt}$ is the flux.

Element fate calculation

We used Equation 15 to calculate element usage. Here, Con^j_i represents element i (includes C and N) usage concentration in j pathway (storage, respiration, biomass, N_2 fixation). We calculated it by summing up the element usage in every time step, which is calculated by F^j_i (the changing rate of element i in j pathway, e.g., C storage rate, C changing rate in respiration, C changing rate in growth) multiplied by the time step Δt.

$$Con^j_i = \sum\left(F^j_i \times \Delta t\right) \quad \text{(Equation 15)}$$

Equation 16 represents the calculation of the percentage of element usage. Here, Per^j_i represents the percentage of j pathway (storage, respiration, biomass, N_2 fixation) of element i (includes C and N) usage. Con^{Total}_i means the total element i usage.

$$Per^j_i = \frac{Con^j_i}{Con^{Total}_i} \times 100\% \quad \text{(Equation 16)}$$

Model simulation

In this study, we did four simulations. We simulated the model under H1 and H2, and under each hypothesis, we simulated two state switching conditions: a 1-min switch and a 6-h switch. All of these happened in 12 h of daytime with light exposure.

Parameters

All parameters we used are included in the supplementary material (Table S1). Most required parameters are obtained or adapted from a previous *Trichodesmium* modeling study,[3] including initial elemental concentrations, environmental O_2 levels, half saturation concentrations, light intensity and coefficient, elemental ratio in biochemical reactions, maximal photosynthesis, N_2 fixation and growth rates, etc. We show these parameters in detail in Table S1.

QUANTIFICATION AND STATISTICAL ANALYSIS

O_2 concentrations dynamics (Figures 2A and 2B) were calculated from Equations 8 and growth rates (Figures 2C and D) were calculated from Equation 6. The element allocation comparison (Figure 3) was calculated from Equations 15 and 16. All the simulations and calculations were completed by using Python 3.8.8 in Eclipse.